TotemBioNet Enrichment Methodology: Application to the Qualitative Regulatory Network of the Cell Metabolism

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Abstract: When designing a biological regulatory network, new information or wet experiments can require adding variables or interactions, inside a previously validated model. They can result in complete reconsiderations of established behaviours. Fortunately, formal methods allow for fully automated verification of properties, and *TotemBioNet* is an efficient software integrating a collection of formal approaches for regulatory networks. It allowed us to develop a multidisciplinary methodology for designing large dynamical models in an incremental way, including non regression proofs (preservation of important biological properties).

1 INTRODUCTION

In the '70s, *qualitative modelling* of biological regulatory networks (Thomas, 1973; Glass, 1975) has led to significant advances in the understanding of the main causalities of some observed cell behaviours. Models were handmade, and parameter identification mainly used simulations (Gonzalez et al., 2006). In the early 2000s, *formal methods* automated the identification of parameters, managing as a whole the *exhaustive sets* of suited parameter settings (Bernot et al., 2004). Software platforms handling formal methods (Khalis et al., 2009; Batt et al., 2004; Paulevé, 2017) have made it possible to design regulatory graphs, where numerous parameter values are compatible with the biological knowledge, where simulations are helpless, and where the slightest modification can have huge consequences (Gebser et al., 2010; Khoodeeram et al., 2017; Naldi et al., 2018).

Enrichments of models do not preserve previously established properties (Bernot and Tahi, 2009; Siebert, 2009; Mabrouki et al., 2011). Nonetheless, in practice, the only way to design big regulatory models is to enrich previously validated models. So, we are moving into an era where intensive verifications of formal properties play the role that simulations played for handmade models: Model revision becomes the current practice (Gouveia et al., 2018). We describe here a methodology to efficiently design large validated models by successive small enrichments of previously validated ones. It uses *TotemBioNet*, a software that offers optimized management of the *exhaustive* set of parameter values compatible with biological knowledge (Boyenval et al., 2020). Here, we describe our methodology and we give a (very small but representative) example of enrichment.It starts from one of the largest regulatory network model using the multivalued Thomas framework (Snoussi, 1989), namely the generic *regulation of the cell metabolism* (Khoodeeram et al., 2017). It abstracts the relationships between the main actors of the metabolism in such a way that, for example, the Crabtree/Warburg effects can be studied, and their main causalities understood. The model considers biomass as a whole, without distinguishing between nucleic acids, proteins or lipids. Here, we simply show how to distinguish between lipidic and non lipidic biomass, so that the enrichment example is small enough to focus on the *TotemBioNet* method rather than describing detailed biological regulations.

Section 2 reminds the Thomas approach and the formal methods implemented in *TotemBioNet*. Section 3 presents our methodology, including the role of different kinds of behaviour "property matrices" that greatly aid decision making for model design. Section 4 shows the illustrating example.

2 *TotemBioNet* RATIONALS

TotemBioNet assists the design of R. Thomas' qualitative regulatory network models. It handles *all* the possible parameterizations, which is the key point of the modelling process.

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2.1 Thomas' Modelling Framework

A regulatory network is a labelled directed graph in which vertices are *variables* (represented by circles) or *multiplexes* (rectangles). Variables abstract biological entities (gene products, biological functions or metabolic pathways) and multiplexes contain formulas that encode situations where a group of variables (inputs of multiplexes) influence the evolution of some variables (outputs of multiplexes), the simplest multiplexes being the formations of complexes (the formula contains a simple conjunction). In Fig. 1 the multiplex `PBM` abstracts the biomass production, it targets variables **`BM`** (BioMass) and **`ATP`**. It is activated when at least one of the multiplexes `AAS`, `LS` or `PPP` is activated. Among others, `AAS` abstracts the amino acid synthesis process. It summarizes the elements necessary for the *de novo* production of amino acids (Berg et al., 2002): nitrogen and carbon donors ($DNC \geqslant 2$), a large amount of NADH ($NADH \geqslant 2$) and ATP ($ATP \geqslant 1$) (necessary for these anabolic processes). This can be expressed by the following formula: $(DNC \geqslant 2)\&(ATP \geqslant 1)\&(NADH \geqslant 2)$.

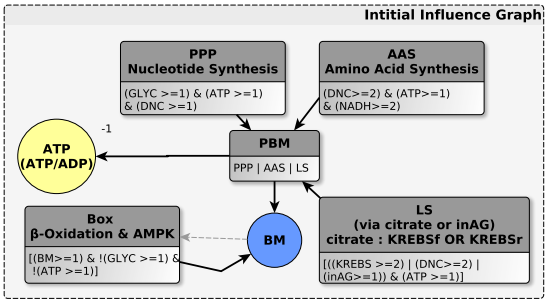

Figure 1: A part of influence graph: representation of variables and multiplexes (focus on **`BM`** in Figure 5).

Each variable has a domain of variation made of a small number of discrete levels and the dynamics of the model is deduced from the regulatory network and a set of *kinetic parameters* $K_{...}$ (simply "parameters" in the sequel). Formally, the presence of an activator or the absence of an inhibitor of a variable x is called a *resource*, and the exhaustive list ω of its resources defines the discrete level towards which x is temporarily attracted, denoted $K_{x,\omega}$. If x has p possible resources then it has 2^p parameters. Lastly, when the set of effective resources of a variable increases, this cannot lead to decreasing its level (Snoussi, 1989): $\omega \subset \omega' \Rightarrow K_{x,\omega} \leqslant K_{x,\omega'}$, which can be used to reduce the number of parametrisations to consider.

2.2 Selection Technics

`TotemBioNet` inherits from SMBioNet (Bernot et al., 2004) and combines two filtering approaches to identify the exhaustive set of models consistent with biological knowledge: "genetically modified Hoare logic" and temporal logic.

Hoare Logic. Biological experiments prove that a set of traces must exist in the model. The genetically modified Hoare logic (Bernot et al., 2019; Folschette, 2019) produces the constraint on parameters that characterizes the models in which these traces exist. We firstly transcript the observed experimental traces into a so-called Hoare triple (observed pre-condition, path and post-condition) and Hoare logic constructs the *weakest pre-condition* that must be fulfilled. This constraint throws away parameter values which do not enable the observed traces.

Temporal Logic. Most of biological knowledge do not translate directly into Hoare logic, such as epigenetic phenomena, homeostasis, (non-)reachability of certain states, some events that always happen after others (but not necessarily right after), etc. To formalize this general knowledge, it is preferable to use temporal logic. (Bernot et al., 2004; Goldfeder and Kugler, 2019) and several other authors have chosen CTL due to the efficiency of its model checking algorithm, and its ability to capture the non-deterministic choices of Thomas' theory.

Mixing Both Approaches. `TotemBioNet` inputs are: an influence graph, any knowledge on the parameter values, and properties on the dynamics of the system expressed using CTL or Hoare logic. It first computes the weakest pre-condition (wp) using genetically modified Hoare logic. Then, it efficiently enumerates all parametrisations satisfying wp, and generates input files for the model checker *NuSMV* (Cimatti et al., 2002). Each file contains the conjunction of CTL formulas and an automaton that encodes the model for the considered parametrisation.

`TotemBioNet`[1], comes with many examples, including those of the present paper. It allows one to describe the influence graph with *yEd* graph editor[2]. A typical session consists in building the influence graph using *yEd*, in automatically generating the associated input file and then adding temporal properties and Hoare triples. `TotemBioNet` generates all parametrisations, labelled with "OK" when the dynamic properties are verified, and if not with all the properties which are not satisfied (see Fig. 2.).

[1] `https://gitlab.com/totembionet/totembionet`
[2] `https://www.yworks.com/products/yed`

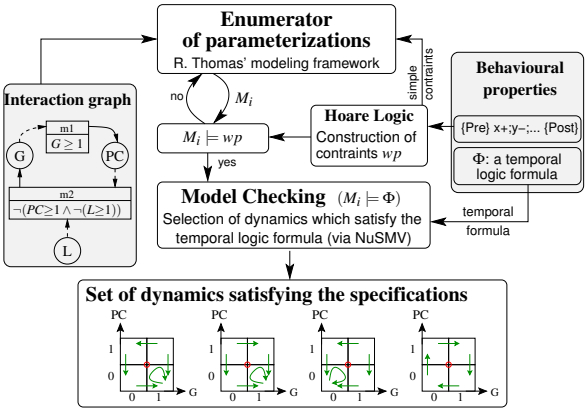

Figure 2: `TotemBioNet` processing flow (Boyenval et al., 2020).

3 *TotemBioNet* ENRICHMENT FRAMEWORK

3.1 Modelling Context

We proceed by successive small enrichments of the model under design. At each step, we start from a regulatory network model together with a *set* of parameter settings (non empty but of reasonable size) that is considered relevant w.r.t. biological knowledge and expertise. For the new enrichment, it is essential to keep track of the fundamental properties of the previously designed network models and to guarantee that they are preserved (possibly reformulated or enriched). Our `TotemBioNet` enrichment framework relies on a *Modelling context*, formed by:

IG: An *Influence Graph* that describes the influence of each variable and multiplex in the system,

BK: Some *Biological Knowledge* on the dynamics of the system, collected from biologists and literature, and given in *natural language*,

PM: A *Property Matrix* that formalizes, (`CTL` or Hoare logic) the dynamics under different conditions. These formalisations are a translation of ***BK***,

PS: A set of *Parameter Settings* that govern system's dynamics. Some of them may be identified from ***BK*** by the modeller, others are automatically selected by `TotemBioNet` to satisfy ***PM*** formulas.

The couple (***BK***,***PM***) is the cornerstone for the reliability of the enrichment process: all along this process, ***PM*** is updated according to ***BK***, and formal verification methods are applied to ***PM***.

3.2 Property Matrices

Each column of a Property Matrix (Fig. 3-top) is a *variable*[3] whose behaviours are described in the column. Each row is an *experimental condition* and a box in the matrix can be filled by a conjunction of `CTL` formulas and Hoare triples that formalizes ***BK***. The experimental conditions are fixed via a set of *environment variables* which are sources of regulations but never target of a regulation. Their constant values can differ from one experimental condition to another.

Along our enrichment workflow, Property Matrices (***PM***) have two different usages, *validation* or *prediction*, as detailed below:
(1)*Validation Property Matrices* (Fig. 3-middle) are ***PM*** where the biologist has certainty on the properties, via literature or wet experiments, and the modeller is confident in the translation into logical formulas. When an enrichment is done, all the properties of the enriched version of a *Validation* ***PM*** must be satisfied. By analogy with software engineering, a *Validation* ***PM*** can be seen as a set of *non regression tests*, (2)*Prediction Property Matrices* (Fig. 3-bottom) focus on fixed parameter settings. They are ***PM*** whose properties have been automatically generated and validated with `TotemBioNet` from simple property patterns such as oscillations, homeostasis, attraction basins and so on. There are as many prediction property matrices as parameter settings: a subset of selected prediction property matrices can then become the starting point of a new enrichment process.

Fig. 3 shows a schematic vision of the usage of ***PM***s along an enrichment of a model, where some variables have been added/removed. A *Validation* ***PM*** may be sparse, as the set of established biological properties is generally small. At the opposite, a *Prediction* ***PM*** is entirely filled, but some of the discovered properties can be of little relevance. This is why the multidisciplinary dialogue is crucial and the increments of ***BK*** are often suggested by the prediction ***PM***s, possibly asking for new biological experiments.

3.3 Enrichment Workflow

Initial Model. The `TotemBioNet` enrichment workflow starts with an initial modelling context (top of Fig. 4). It contains a *Validation* ***PM*** that will be enriched. The challenge is to design an enriched modelling context that ensures non regression w.r.t. the initial validation matrix.

Enrichment Design. The design of an *enriched modelling context* lies on a dialogue between the biologist and the modeller (Fig. 4-middle). The biologist

[3]Or possibly a few variables

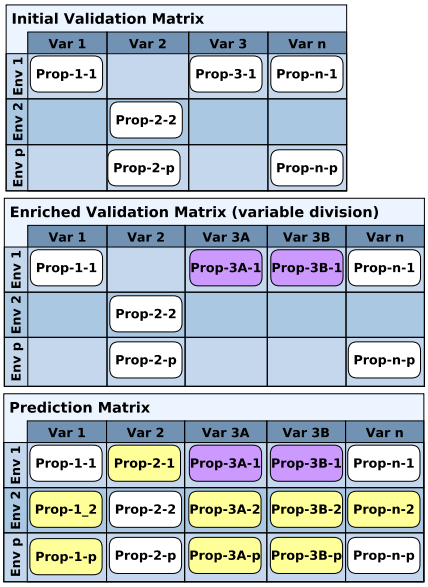

Figure 3: Schematic vision of Property Matrices (***PM***). Top: initial validation ***PM***. Middle: enriched validation ***PM*** where ***var-3*** has been divided into `var-3A` and `var-3B` leading to the generation of purple properties `prop-3A-1` and `prop-3B-1` from the initial property `prop-3-1`. Bottom: prediction ***PM*** where yellow properties have been automatically generated.

describes the *Enriched Biological Knowledge* (***EBK***) and the modeller propagates it by enriching the *Influence Graph* (***IG***) or the *Property Matrix* (***PM***) (they become ***EIG*** and ***EPM***). A modification of ***IG*** may induce a modification of the validation matrix, for example by changing the level of an homeostasis. It may also induce new parameters, thus `TotemBioNet` is run to identify the unknown parameters that validate ***EPM***. If there is no such parameters, the modelling is inconsistent and has to be corrected. If there are too many parameter settings, the modelling is not precise enough to be a good candidate for predicting new relevant biological properties (Occam's razor). Thus the process goes back to a dialogue for refining the model. When a reasonably small number of parameter settings validate ***EPM***, the biologist takes the final decision: return to improve the models and design a better enrichment, or continue the workflow.

Test and Validation of an Enriched Modelling Context. When a set of parameter settings has been selected as the best compromise, tests and validations begin (Fig. 4-bottom). An *instanciated* modelling context is then built for *each* parameter setting. The dynamic behaviours controlled by these settings ensure a non regression of the initial modelling, but they may also exhibit some behavioural properties that differ from one model to another. Thus the *matrix completion* functionality of `TotemBioNet` is called on *each instantiated* model. `TotemBioNet` establishes a set of properties on each variable in each context[4], which are added to the matrix, as predictions. Lastly, the biologist and the modeller select the most interesting contexts according to phenotypes, possibly with the help of new biological experiments. This set of contexts is the starting point of another enrichment.

4 ILLUSTRATING EXAMPLE

This section illustrates the enrichment steps of Fig 4. The cell metabolism can be viewed as an energy balance where all metabolic pathways (linked series of chemical reactions) are regulated according to nutrient cell intake. They can be anabolic (producing biomass compounds) and catabolic (that degrade large compounds to produce energy). The anabolic and catabolic balance is fine-tuned in the cell.

Within this context, an abstract and qualitative model of the metabolism regulation was developed (Khoodeeram et al., 2017) to understand how activity level of biological pathways in combination with nutrient can influence Warburg/Crabtree effect. This effect appears in all cell types from yeast to human: It leads the cells to go from one catabolic process (respiration) to another (fermentation). Since 2017, in order to focus on cancer in human cells in correlation with the cell cycle, we made several enrichments of the model to better reflect key metabolic pathways (glycolysis, oxidative respiration, Krebs cycle), nutrient (oxygen or glucose), key molecular components (biomass) and regulators (key-cofactors).

The following subsections correspond respectively to the top, middle and bottom parts of Fig. 4.

4.1 Initial Model and its Limits

The enrichment starts from the model whose ***IG*** is given in Fig. 5: biomass was represented by a unique variable **`BM`** that covered all cell growth components (lipids, nucleic acids for genetic material and amino acids for proteins). Fig. 1 is an extract from Fig. 5 that focuses on **`BM`**: the multiplexes `PPP`, `AAS` and `LS` abstract anabolic pathways respectively producing nucleic acids, amino acids and lipids (Axelrod, 1967; Berg et al., 2002; Stein and Stein, 1967). If one of them is activated, biomass production (`PBM`) is on: `PBM` is the inclusive disjunction of `PPP` and `AAS` and

[4]Currently, only oscillations, homeostasis, and stable states are implemented in prediction property matrices but enriching this range of properties is not difficult.

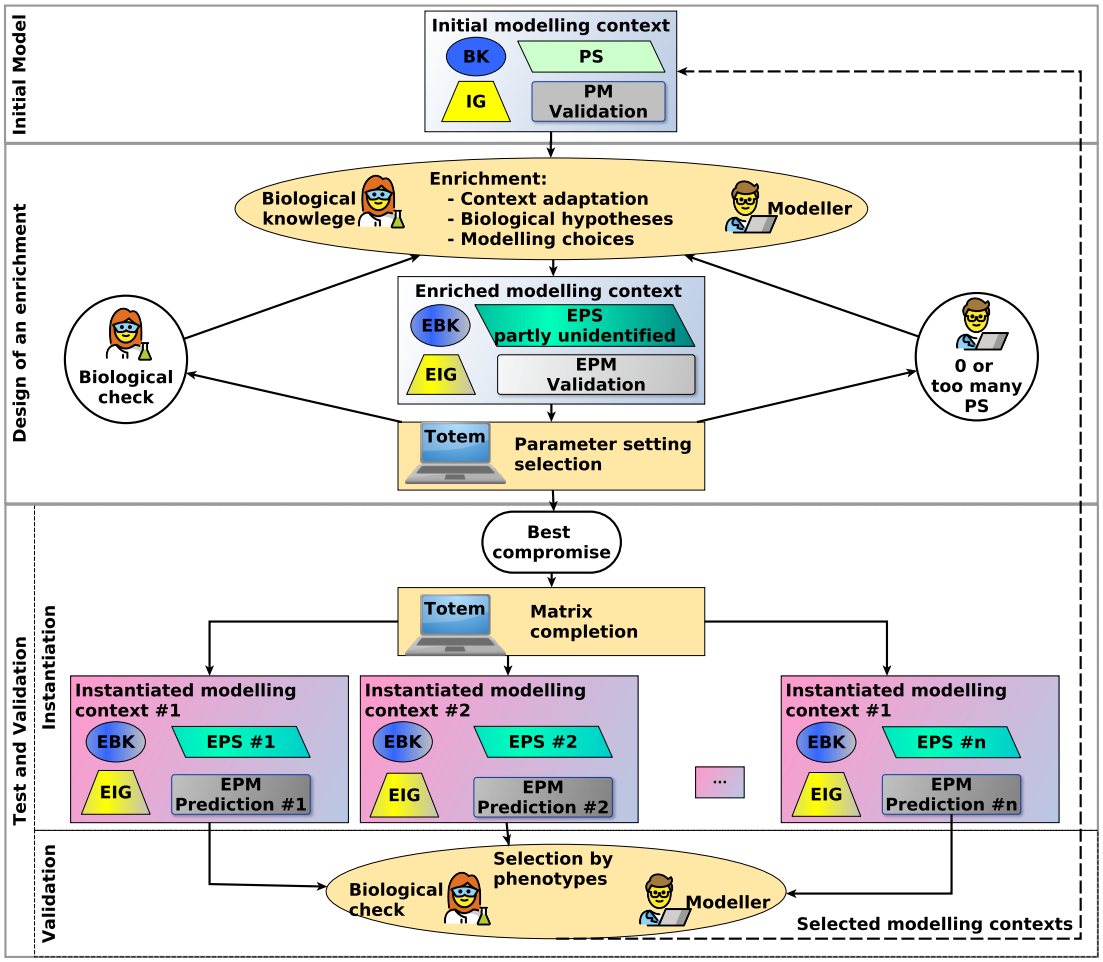

Figure 4: `TotemBioNet` Enrichment Framework. The quadruplet of a *modelling context* is represented by a box containing: a yellow trapeze (***IG***), a blue bubble (***BK***), a grey rectangle (***PM***) and a green parallelogram (***PS***). The prefix ***E*** means an Enrichment of one part of the quadruplet.

`LS` $(AAS|PPP|LS)$. When `PBM` is a resource of **BM**, biomass will become present in the cell[5].

Table 1: Subpart of the Initial validation matrix. "0" means *tend toward 0*, "Osc" means *oscillations* with arbitrary boundaries and "!" stands for the *negation*.

Initial validation matrix							
Environment			*Behaviour*				
OXYG	GLC	IN_AA	ATP	BM	FERM	KREBS	*PHOX*
0	*0*	*0*	*0*	***0***	*0*	*0*	0
0	*1*	*0*	*Osc*		*!0*		0
1	*1*	*1*	*Osc*	***!0***	*0*	*Osc*	Osc
1	*2*	*2*	*Osc*	***!0***	*!0*		Osc

Table 1 is a subpart of the validation matrix of this initial model. The column **BM**, for example, requires obvious properties, e.g. absence of biomass if the cell does not receive any nutrient, and so on.

[5]Because the applicable parameter is $K_{BM,PBM} = 1$ in the current parameter setting

BM participates in `Box`, which abstracts the β-oxidation pathway, referring to the catabolic process of lipids (Wakil, 1970). Unfortunately, this ***IG*** implies that β-oxidation could be activated without any lipids input. Indeed `Box` is satisfied when **BM** is activated by `PPP` (nucleic acids) or `AAS` (amino acids) through `PBM`: this behaviour proves that considering biomass as a whole is a too coarse abstraction for an adaptation to human cells. So, separating lipid biomass from the other compounds is our next enrichment.

4.2 Enriched Modelling Context Design

EBK: Enriched Biological Knowledge. It lists all biological information needed to separate lipids from the rest of the biomass: lipids are produced by synthesis and degraded by β-oxidation. Amino acids synthesis produces proteins, and PPP (Pentose Phosphate Pathway) produces nucleic acids for genetic material.

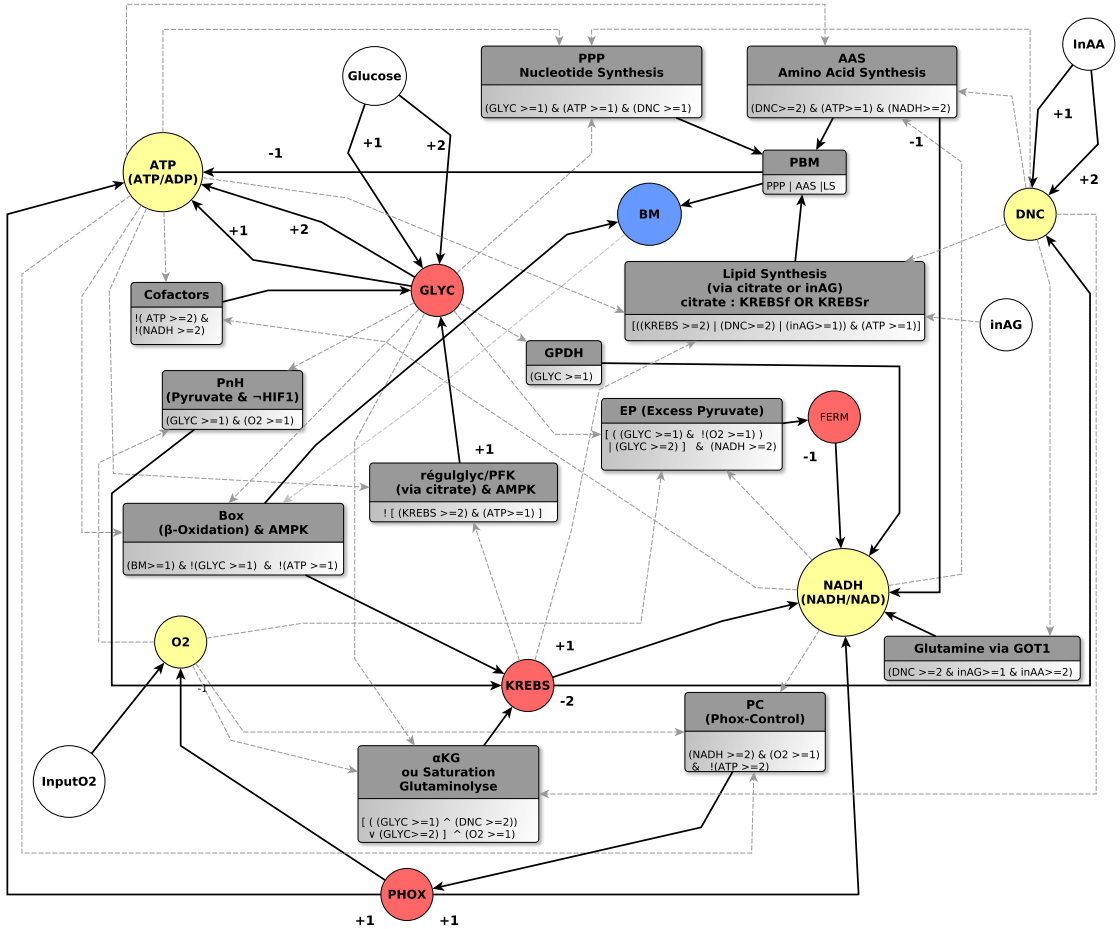

Figure 5: Influence graph (***IG***) of the initial modelling context: variables in red are the main metabolic processes (glycolysis, Krebs cycle, oxydative phosphorylation), yellow ones are the main cofactors (ATP/ADP and NADH/NAD+ ratios, oxygen, amino acid donors), biomass is in blue, environment variables are white (oxygen intake, glucose, nutrients...) and rectangle multiplexes formalize their interactions. For more details see (Khoodeeram et al., 2017) who defined the first ***IG*** of the cell metabolism regulation and its whole parameter setting.

Each kind of biomass production needs *ATP* for energy. All of this is added to initial ***BK*** to obtain ***EBK***.

EIG: Enriched Influence Graph. Accordingly, we modify Fig. 1 into Fig. 6. It focuses on what has been modified: the biomass variable and its surrounding multiplexes. In the ***EIG*** (Fig. 6) **`BM`** has been split into **`BL`** (lipids biomass) and **`BnL`** (non lipidic compounds). **`BL`** participates in `Box` and is regulated by `LS`. **`BnL`** resources are `PPP` and `AAS`. In the multiplex `Box` the variable **`BM`** is replaced by the variable **`BL`**, which solves the problem mentioned in Subsection 4.1. Lastly, both **`BL`** and **`BnL`** productions consume **`ATP`**, thus regulate it negatively.

EPS: Enriched Parameter Settings. Our ***EIG*** leads to new parameters that need to be identified. **`BL`** and **`BnL`** have two resources each, so there are $(2^2 + 2^2 = 8)$ new parameters (see Section 2.1). Moreover **`ATP`** gains one resource (5 resources in the ***EIG*** instead of 4 in ***IG***). It increases the number of **`ATP`** parameters to $2^5 = 32$. Among them, 16 are ineffective due to unsatisfiability of some resource combinations, and the 6 identified parameters which do not involve new resources are kept. Finally, it remains 18 parame-

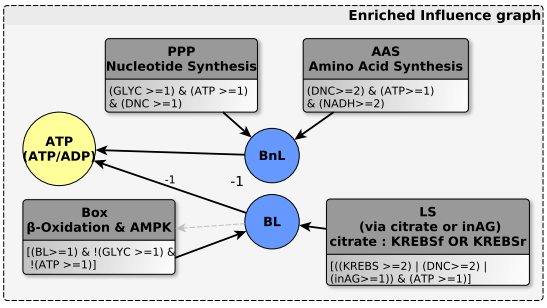

Figure 6: Enrichment of the influence graph of Fig. 1: **`BM`** has been split into **`Bnl`** and **`BL`**.

ters to identify for **ATP**. Thus, we have 26 parameters to identify.
Thought experiments can be useful. For instance, to identify the parameter $K_{BnL,\varnothing}$, we assume that both pentose phosphate pathway (PPP) and amino acids synthesis (AAS) are inactive (the set of **Bnl** resources is $\varnothing$). We try to foresee toward which value would **BnL** go if these conditions persist: neither genetic material nor proteins are produced, thus $K_{BnL,\varnothing} = 0$. Most of the time ***EBK*** is not sufficient to allow the parameter identifications (many thought experiments are inconclusive). In such cases TotemBioNet will test all possible parameter settings. All in all 5 out of the 26 parameters were not identified.

EPM: Validation Enriched Properties Matrix. It summarizes the dynamical behaviours of variables, according to ***EBK***. The only modifications affect **BM** (Table 2). It is replaced by both columns **BnL** and **BL** and they are filled from ***EBK***: For example without any intake of glucose, no compounds are synthesized so **BnL** and **BL** tend towards 0.

Table 2: Subpart of the validation ***EPM***.

Enriched validation matrix								
Environment			***Behaviour***					
OXYG	GLC	IN_AA	ATP	BnL	BL	FERM	KREBS	*PHOX*
0	*0*	*0*	*0*	***0***	***0***	*0*	*0*	0
0	*1*	*0*	*Osc*			*!0*		0
1	*1*	*1*	*Osc*	***!0***	***!0***	*0*	*Osc*	Osc
1	*2*	*2*	*Osc*	***!0***	***!0***	*!0*		Osc

4.3 TotemBioNet Test and Validation

We test with TotemBioNet the enriched modelling context created above, taking into consideration ***EBK, EIG, EPS, EPM***. The 5 unidentified parameters from ***EPS*** give rise to only 15 *instantiated* modelling contexts (where each parameter has a unique value). We obtain 15 prediction matrices such as Table 3 where all empty boxes of validation-EPM are automatically completed using TotemBioNet. They allow modellers and biologists to eliminate parameter settings which exhibit non credible behaviours.

Table 3: Sub part of one of the 15 prediction ***EPM***s."Osc(0-1)" means *oscillations* between level 0 and 1 of the variable.

Enriched Prediction matrix								
Environment			***Behaviour***					
OXYG	GLC	IN_AA	ATP	BnL	BL	FERM	KREBS	*PHOX*
0	*0*	*0*	***0***	***0***	***0***	***0***	***0***	**0**
0	*1*	*0*	***Osc(0-2)***	***Osc(0-1)***	***0***	***Osc(0-1)***	***0***	**0**
1	*1*	*1*	***Osc(0-2)***	***Osc(0-1)***	***Osc(0-1)***	***0***	***Osc(0-1)***	**Osc(0-1)**
1	*2*	*2*	***Osc(0-2)***	***Osc(0-1)***	***Osc(0-1)***	***Osc(0-1)***	***Osc(0-2)***	**Osc(0-1)**

For our example, the 15 prediction ***EPM***s exhibit interestingly different behaviour for the variables **BL** and **BnL**. Here, none of the models have aberrant predictions, so we keep this enriched modelling context for the next enrichment step. Nevertheless, in general some of these different behaviours can contradict established biological knowledge or suggest new biological experiments.

5 CONCLUSION

We defined an incremental methodology for developing large formal models of biological regulatory networks. Starting from an initial formal model, the enrichment is first described in natural language ***EBK***, then the formalisation of this information leads to the ***EIG***, ***EPS*** and validation ***EPM***. Our process involves, at each round of this methodology, a manageable number of parameter settings.

In practice, we observe that after a few enrichment steps, the ab initio design of the enriched model, without using the proposed methodology, would be impossible. The example outlined in this article (that contains notably more than 100 parameters) would lead to a number of parametrizations (1.45×10^{60}) that is so huge that TotemBioNet or any similar platform would have taken more that 4.6×10^{50} years of computation to model check them, at the rate of 100 proofs per second! One of the key points of our incremental methodology consists in limiting, at each enrichment step, the number of parameter settings to enumerate, preserving a maximum number of known parameters from the previously validated model. ***EPS*** and validation ***EPM*** in an *enriched modelling context* allow TotemBioNet to drastically reduce the number of total parameters to identify, with a negligible computation time: The parameters for which the resources do not change (according to the new interpretation) remain at the same value, and the new ones can partly be identified by thought experiments.

Moreover, following our methodology, getting too many parameter settings for an enriched modelling context, simply means that the enrichment step is too ambitious to be manageable. In such a case we reassess the informal enrichment ***EBK*** to address a less ambitious enrichment. Conversely, when no coherent parameter setting is obtained, this is generally due to a construction error in the enriched modelling context which has also to be reassessed. In our methodology, this loop of reassessment is made possible owing to the remarkably efficient TotemBioNet platform.

We currently use this methodology for the metabolism regulation in cancer cells, when cells acquire new functioning during the Epithelial-mesenchymal transition. In practice, the informal ***BK*** plays a crucial role for assisting interactions between biologists and modellers, not only in the design of the enrichment but also, in order to keep track of interpretations made in previous contexts. Last but not least,

at each step the choice of a sensible subset of parameter settings often suggest new biological experiments that are particularly revealing.

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